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Molecular weight of polyanion affects the biological activity of interpolycomplexes

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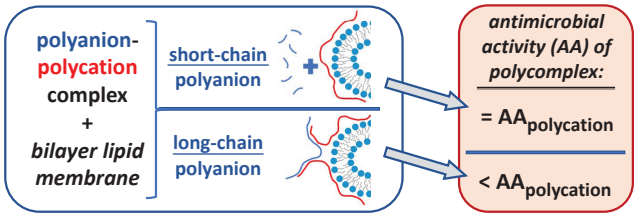
Cationic poly(diallyldimethylammonium chloride) forms nonstoichiometric positively charged interpolyelectrolyte complexes (IPECs) with short- and long-chain anionic sodium polyacrylate. When added to anionic liposomes, the short-chain polyanion IPECs dissociate and the free polycation binds to the liposomes, whereas the long-chain polyanion IPECs bind to the liposomes as a whole. These results correlate with the antimicrobial activity of IPECs, thereby highlighting the important role of polymer molecular weight in the cellular response to IPEC binding.



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Polymers with cationic groups (polycations) are used as immunostimulants,[1] delivery vehicles for genetic material,[2] anticoagulants,[3] antifungal and antibacterial agents.[4,5] Modification of polycations with anionic polymers leads to the formation of interpolyelectrolyte complexes (IPECs), which are compounds with movable hydrophobic fragments represented by mutually neutralized charges of both polymers and loops and tails consisting of separated hydrophilic polycation and polyanion units.[6] Nonstoichiometric IPECs (NIPECs) with an excess of any component are soluble in water.[6,7] Hydrophobic blocks increase the affinity of the whole construct for the biological membrane and can incorporate drugs, thereby enhancing their biological effect.[5,8,9]

These findings have stimulated studies of ionic polymers (polyelectrolytes, PEs) in biological environment and especially their interactions with cells. In these studies, along with native cells, cell-mimetic objects, spherical bilayer lipid vesicles (liposomes), were used.[9,10] The structure of the lipid bilayer in the liposomal model is very close to the structure of the cell surface and the liposome surface can be fully characterized.[11,12] It has been shown that the binding of PE to liposomes is accompanied by the incorporation of PE into the liposomal membrane and an increase in membrane permeability,[9,13] lateral segregation of lipids and transmembrane migration of lipid molecules (flip-flop),[14] aggregation, fusion and disruption of liposomes.[15–17] These effects, if they occur in a biological membrane, can affect the functioning of cells. The situation with NIPECs is more complicated. When interacting with liposomes, NIPECs can dissociate or bind as a whole; the mechanism of this key stage determines the entire chain of events, starting with the coupling of NIPECs and ending with the effect of NIPECs on the integrity of the biological membrane.

In this paper, we describe two types of NIPECs with an excess of cationic units ('cationic' NIPECs). In the first type, cationic charges are partially neutralized by a short-chain polyanion, the degree of polymerization (DP) of which is significantly lower than the DP of the polycation, and in the second type the DPs of both PEs were comparable. We show that these polycomplexes interact differently with anionic liposomes, and this difference affects the state of the liposomal membrane. Additionally, we discuss how bacterial cells respond to the addition of both types of NIPECs, thereby combining model and cell-based approaches.

Separate solutions of cationic poly(diallyldimethylammonium chloride) (PDADMAC) with M_w = 470 kDa and anionic sodium polyacrylate (PANa) with M_w = 8 kDa (PANa1) or M_w = 250 kDa (PANa2) in 1 mM Tris buffer (pH 7) were mixed to obtain IPEC,[18,19] which was detected using microelectrophoresis and dynamic light scattering (for details, see Online Supplementary Materials). As an example, Figure 1(*a*) shows two curves that reflect changes in the electrophoretic mobility (EPM) of PDADMAC upon binding to PANa1 and PANa2. Here and below, polymer concentrations are shown in moles of monomer units per liter, cationic for PDADMAC, [N^+], and anionic for PANa, [COO^-]. In both cases, binding resulted in a gradual decrease in the PDADMAC charge down to EPM = 0, *i.e.*, to complete neutralization of the positive charge of PDADMAC by the negative charge of PANa.

In parallel, the size of IPEC complexes was measured by dynamic light scattering. Figure 1(*b*) shows a slight change in hydrodynamic diameter as Z = [PANa]/[PDADMAC] increases from 0 to 0.4. For further experiments, IPECs with $Z \leqslant 0.4$ were used. Taking into account the M_w of the polymers involved in complex formation, the resulting IPEC can be represented as

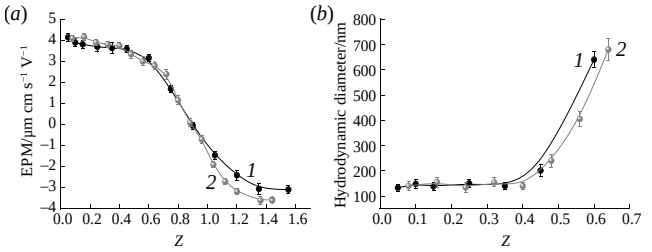

Figure 1 Dependences of (*a*) EPM and (*b*) hydrodynamic diameter of PDADMAC–PANa on the ratio $Z = [COO^-]/[N^+]$ at $[N^+] = 5$ mM for polyanions (*1*) PANa1 and (*2*) PANa2 in 1 mM Tris buffer (pH 7) containing 10 mM NaCl. Bars represent standard deviations from the mean.

follows. Cationic IPEC with short-chain PANa1 arises from the binding of a single PDADMAC chain to several polyanionic chains (Figure 2, part *1*). In contrast, cationic IPEC with long-chain PANa2 can be drawn as a single polyanionic chain bound to multiple PDADMAC chains (Figure 2, part *2*). This idea of the structure of the polycomplex is based on previously published works that describe the complex formation of two oppositely charged polyelectrolytes.[6,7,20,21]

An increase in *Z* above 0.4 was accompanied by a sharp rise in particle size due to pronounced aggregation of IPECs particles [see Figure 1(*b*)]. An increase in the size of IPEC particles upon sequential loading of a linear polyelectrolyte with an oppositely charged polyelectrolyte has also been noted in published works.[22–24] Aggregation is usually associated with mutual neutralization of the charges of both polymers,[6,7,23] with the largest aggregates found in the range close to EPM = 0.

According to published works,[6,7] the oppositely charged polyanion and polycation quantitatively bind to each other until a stoichiometric IPEC is formed. This conclusion is obviously valid for the complexation of PDADMAC with PANa. Thus, the *Z* value reflects not only the ratio of PDADMAC to PANa in the reaction mixture, but also the composition of the resulting cationic IPECs. It is the abundant positive charges of IPECs that ensure their stability against aggregation in water salt solutions.

To study the interaction of IPECs with liposomes, solutions of IPECs with *Z* values ranging from 0.1 to 0.4 were added to a suspension of 85 nm diameter liposomes consisting of anionic palmitoyloleoylphosphatidylserine ($POPS^{1-}$) and zwitterionic dioleoylphosphatidylcholine (DOPC), with a mole fraction of anionic $POPS^{1-}$ $Q = 0.2$, where *Q* is given by $Q = [POPS^{1-}]/\{[POPS^{1-}] + [DOPC]\}$ (see Online Supplementary Materials). The interaction of IPECs with liposomes was monitored by measuring the EPM of the particles in the system. Figure 3(*a*) shows the EPM values for the cases of the initial PDADMAC and two IPECs with minimum ($Z = 0.1$) and maximum ($Z = 0.4$) content of short-chain PANa1, with the *x*-axis in this figure indicating the molar concentration of cationic PDADMAC groups. The addition of PDADMAC and cationic PANa1-based IPECs resulted in neutralization of the liposome charge, with EPM = 0 being achieved at a PDADMAC concentration of $(2.8 \pm 0.02) \times 10^{-4}$ M in all three cases.

A complete list of neutralizing concentrations of PDADMAC for IPECs with *Z* in the range from 0 (when adding only PDADMAC) to 0.4 is presented in Table 1.

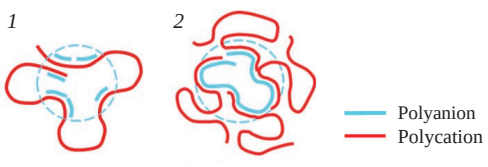

Figure 2 Schematic representations of cationic IPECs with (*1*) short-chain PANa1 and (*2*) long-chain PANa2.

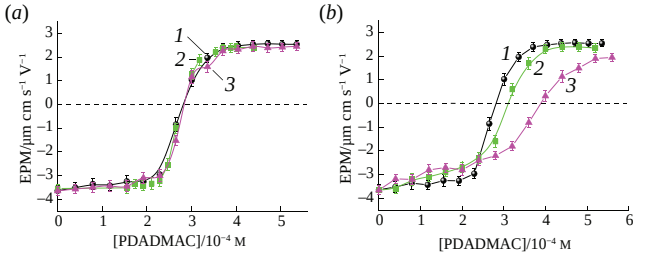

Figure 3 Dependences of the EPM of (*1*) liposome/PDADMAC and (*2*),(*3*) liposome/IPEC on the concentration of PDADMAC in 1 mM Tris buffer (pH 7) solution at a total lipid concentration of 1 mg ml^{-1} for (*a*),(*b*) initial PDADMAC, as well as for IPECs based on (*a*) PANa1 and (*b*) PANa2, with (*1*) $Z = 0$, (*2*) $Z = 0.1$ and (*3*) $Z = 0.4$. Bars represent standard deviations from the mean.

Table 1 Neutralizing concentrations of PDADMAC (C_0) upon binding of PDADMAC, PANa1-based IPECs and PANa2-based IPECs to $POPS^{1-}$/DOPC liposomes.

Sample	*Z*	$C_0/10^{-4}$ M	
		PANa1	PANa2
PDADMAC	0	2.81	2.81
PDADMAC–PANa	0.1	2.8	3.1
PDADMAC–PANa	0.2	2.82	3.3
PDADMAC–PANa	0.3	2.81	3.53
PDADMAC–PANa	0.4	2.84	3.88

This means that the adsorption of individual cationic PDADMAC and both cationic PANa1-based IPECs follows the same pattern, in which all added polycations bind to the liposomes and neutralize the charge of the liposomes. Therefore, the addition of IPECs initiates a competitive reaction, in which PDADMAC, which initially complexed with PANa1, loses the polyanion and forms an electrostatic complex with the anionic liposomes. Thus, in the ternary PDADMAC–PANa1–liposome system, the $POPS^{1-}$/DOPC liposome is a stronger competitor for PDADMAC binding compared to short-chain PANa1.

We now consider the binding of $POPS^{1-}$/DOPC liposomes to IPECs of long-chain PANa2 [Figure 3(*b*)]. With increasing IPEC content, binding also led to a decrease in the negative charge, but the neutralizing concentrations of PDADMAC for the two IPECs differed significantly: 3.1×10^{-4} M for IPEC with $Z = 0.1$ and 3.88×10^{-4} M for IPEC with $Z = 0.4$. Analysis of the full list of C_0 values (see Table 1) showed a progressive increase in C_0 with increasing *Z*. This dependence of C_0 on *Z* clearly indicates that IPECs of long-chain PANa2 do not dissociate during binding to liposomes. The higher the Z value, the more cationic PDADMAC units are electrostatically bound to the anionic PANa2 units and for this reason are not involved in the formation of complexes with liposomes. This is reflected in the shift of the C_0 *vs.* *Z* plot to the right in Figure 3(*b*). Thus, we see quite different behavior of cationic IPECs with short- and long-chain polyanions when binding IPECs to anionic $POPS^{1-}$/DOPC liposomes. The liposomes displace short-chain PANa1 from IPECs and form a binary liposome–PDADMAC complex, releasing PANa1 into solution. In contrast, the IPEC of long-chain PANa2 interacts with liposomes as a whole, resulting in the formation of a ternary PDADMAC–PANa2–liposome complex.

The antimicrobial activity of aqueous polymer formulations was assessed by determining their minimum inhibitory concentrations (MICs) against the gram-negative bacteria *Pseudomonas aeruginosa* 4.8.1 from the collection of the Research Center of Biotechnology of the Russian Academy of Sciences (see Online Supplementary Materials). The antimicrobial activity of IPECs was tested in 0.08 M saline solution. At that time, it was known that IPECs are sensitive to the salt concentration in an aqueous solution. An increase in salt

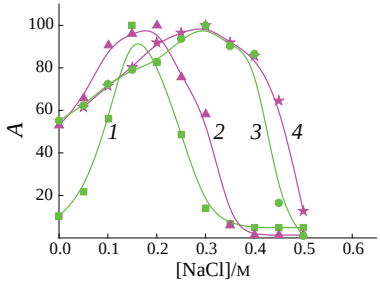

Figure 4 Dependences of the relative optical density (*A*) of IPEC dispersions at 500 nm on the NaCl concentration in 1 mM Tris buffer (pH 7) at [N^+] = 50 mM for IPECs based on (*1*),(*2*) PANa1 and (*3*),(*4*) PANa2, with (*1*),(*3*) *Z* = 0.1 and (*2*),(*4*) *Z* = 0.4.

concentration leads to the dissociation of IPEC down to the initial components, *i.e.*, polycation and polyanion.[7,25] The critical salt concentration leading to quantitative dissociation of IPEC depends on a number of factors, including the chemical nature of the polymers that form IPEC. Taking this into account, the stability of PDADMAC–PANa IPECs in water salt solutions was investigated by measuring the optical density of IPEC suspensions (*A*) in the presence of increasing concentrations of NaCl. However, 5 mM IPEC solutions with $Z \leqslant 0.4$ are almost transparent and for this reason cannot be useful for salt-induced IPEC dissociation experiments. To detect dissociation, the IPEC concentration was increased to 50 mM, while maintaining the IPEC composition with *Z* equal to 0.1 and 0.4, as before.

Figure 4 shows *A vs.* [NaCl] plots for PDADMAC–PANa1 IPEC (curve *1*) and PDADMAC–PANa2 IPEC (curve *2*) in concentrated IPEC suspensions. Increasing the concentration of NaCl in the IPEC solution first led to an increase in turbidity and then to a clear solution at [NaCl] $\geqslant$ 0.35 M, reflecting the dissociation of IPEC. We compared two salt concentrations, a 0.35 M concentration that caused IPEC dissociation and a 0.08 M concentration used in the M9 media, and concluded that IPECs persisted in the antimicrobial experiments and interacted with bacterial cells as a whole.

The results of antimicrobial testing are presented in Table 2. Individual PDADMAC and the PDADMAC–PANa1 IPEC have the same MIC value of $(1.1 \pm 0.1) \times 10^{-3}$ wt%. This coincidence is consistent with the above mechanism for the dissociation of PANa1-based IPECs upon contact with a biological (liposomal) membrane. Dissociation of IPEC releases a polycation that exhibits antimicrobial activity equal to that of individual PDADMAC. The MIC value for the PANa2-based IPEC (1.8×10^{-3} wt%) is two times higher, which indicates a 2-fold decrease in the antimicrobial activity of this sample. This result correlates with the fact that the PDADMAC–PANa2 IPEC retains its integrity after complexation with liposomes.

To summarize, cationic PDADMAC (M_w = 470 kDa) forms nonstoichiometric IPECs with short-chain PANa1 (M_w = 8 kDa) and long-chain PANa2 (M_w = 250 kDa), with IPECs abundantly bearing cationic groups. According to electrophoresis, when added to a suspension of anionic liposomes, PANa1-based IPECs dissociate and the released PDADMAC binds to liposomes, whereas PANa2-based IPECs retain their integrity and bind to liposomes as a whole. These results correlate with the antimicrobial activity of IPECs. Dissociating PANa1-based IPECs exhibit activity comparable to that of the initial PDADMAC. Stable PANa2-based IPECs with a partially neutralized PDADMC charge have lower antimicrobial activity. These results indicate that polymer molecular weight affects the interaction of IPECs with biological membranes and cell functioning. The mechanism of IPEC complexation with biomembranes requires more detailed study. IPECs with controlled composition and properties can be used as carriers for drug delivery, aqueous biocidal formulations and antimicrobial coatings.

Table 2 Antimicrobial activity of polymer formulations in solution.

Polymer formulation	*Z*	MIC (wt%)
PDADMAC	0	$(1.0 \pm 0.1) \times 10^{-3}$
PDADMAC–PANa1 IPEC	0.4	$(1.2 \pm 0.1) \times 10^{-3}$
PDADMAC–PANa2 IPEC	0.4	$(1.8 \pm 0.2) \times 10^{-3}$

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2024.01.029.

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